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NP10 8QQ

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Request for grant of a patent

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1	Your reference	P15770
2	Patent application number	9930252.3 22 DEC 1999
3	Full name, address and postcode of the applicant	ML Laboratories 17 Hanover Square LONDON W1R 9AJ
	Patents ADP number	
	State of incorporation	UK 07115280005
4	Title of the invention	Biologically Active Molecules
5	Name of agent	HARRISON GODDARD FOOTE
	Address for service	Belmont House 20 Wood Lane LEEDS LS6 2AE
	Patents ADP number	14571001 ✓

6	Priority applications	Country	Priority App No	Date of Filing

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7	Parent application (eg Divisional)	Earlier Application No	Date of Filing
8	Statement of Inventorship Needed?	yes	
9	Number of sheets for any of the following (not counting copies of same document)		
	Continuation sheets of this form		
	Description	8	
	Claims		
	Abstract		
	Drawings	3	
10	Number of other documents attached		
	Priority documents		
	Translations of priority documents		
	P7/77		
	P9/77		
	P10/77		
	Other documents		
11	I/We request the grant of a patent on the basis of this application.		
	Signature	Harrison Goddard Foote	22 December 1999
12	Name and daytime telephone number of person to contact in the United Kingdom	**Dr Rob Docherty** **0113 225 8350**	

BIOLOGICALLY ACTIVE MATERIALS

Field of Invention

This invention relates to biologically active materials and, in particular, to materials which comprise a biodegradable polymer linked to a biologically active agent. The invention is concerned with materials known as polymer-drug conjugates which typically contain a therapeutic agent for instance, a bioactive cytotoxic drug, linked to a polymer back-bone. The linkage between the polymer and the drug is typically by covalent bonding. However, the invention is applicable to other polymer conjugates including those where the biologically active agent is an imaging agent, such as tyrosinamide, a diagnostic agent, or a targeting agent such as biotin.

Reference will be made hereinbelow to polymer-drug conjugates in which the drugs are anticancer agents. However, the present invention has application in connection with other drugs and/or bioactive agents.

Background of the Invention

In designing a polymer-drug conjugate, the aim is to deliver a drug effectively to a therapeutic site such as a tumour. It is known, for instance, that polymer-drugs given intravenously can accumulate selectively in solid tumour tissue by the EPR effect.

The most commonly used anticancer agents are low molecular weight compounds which readily gain access to cells by rapid passage across the cell membrane. After intravenous (IV) administration, a large percentage of the injected dose leaves the circulation within a few minutes, resulting in a ubiquitous body distribution of drug and little selective concentration in tumour tissue. By creating a macromolecular polymer-anticancer drug conjugate, there is provided an opportunity to improve tumour specific targeting, to minimise drug entry into sites of toxicity, to control precisely the rate of drug liberation at the target site (giving opportunities for long-

term controlled release) and to deliver the active principal intracellularly, thereby providing a means to overcome p-glycoprotein related multidrug resistance.

Numerous polymers have been proposed for synthesis of polymer-drug conjugates including polyaminoacids, polysaccharides such as dextran, and synthetic polymers such as N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer. However, these polymers have limitations. For example, a dextran-doxorubicin conjugate has been tested clinically and been found to be much more toxic than the parent drug. Furthermore the HPMA copolymers which have been clinically tested have the disadvantage of being non-biodegradable in the main chain.

WO-A-98/56424 discloses a polymer-drug conjugate in which the polymer is the polysaccharide dextrin. Such a polymer-drug conjugate may be prepared in various ways. One method involves succinoylating dextrin and reacting the succinoylated dextrin with the drug or a reactive derivative thereof.

WO-A-98/56424 includes an example in which the extent of succinoylation of dextrin varies from 2.26 to 6.64 Mol%. In a further example the drug doxorubicin is conjugated to succinoylated dextrins in which the extent of succinoylation varies from 0.5 to 14.9 Mol%.

WO-A-98/56424 also includes examples showing the rate of degradation of dextrin both in the absence and in the presence of appropriate enzymes and also in rat plasma.

For at least certain applications the rate of degradation of dextrin in a dextrin-drug conjugate is an important consideration. For instance, it may be desirable to have a relatively slow rate of degradation in some applications while in other applications a faster rate of degradation is either acceptable or indeed even preferred.

Statement of Invention

It has now been surprisingly discovered that the rate of dextrin degradation is highly dependent on the degree of dextrin backbone substitution. As a result, it is possible to tailor the dextrin by appropriate substitution of its backbone in order to achieve a desired rate of degradation.

More particularly, it has been found that, in the case of substitution of the dextrin backbone by succinoylation, relatively rapid degradation takes place at a degree of succinoylation of up to about 15%. By contrast a degree of succinoylation above 30% very markedly reduces the rate of degradation.

The present invention provides a dextrin-drug conjugate in which the degree of substitution of the dextrin chain is greater than 15%, more preferably greater than 20% and most preferably greater than 30%.

The drug of the dextrin-drug conjugate may be loaded on the polymer via a linking group, such as succinoyl, in which case it may be attached to some or all of the linking groups. Alternatively the drug may be directly loaded onto the dextrin backbone in which case the drug itself acts as the substituting group. As a further possibility the drug may be loaded partly via a substituting group and partly directly onto the dextrin backbone.

An embodiment of the invention will now be described by example only and with reference to the following tables and figures;

Table 1 represents the characteristics of different batches of succinolyated dextrin doxorubicin conjugates;

Table 2 shows the anticancer activity of succinolyated dextrin doxorubicin conjugates;

Figure 1 is a graphical representation of the degradation of dextrin, succinoylated dextrin and a succinolyated dextrin doxorubicin conjugate (5% succinolyation, 6% doxorubicin);

Figure 2 is a graphical representation of the degradation of hyper-succinoylated dextrin doxorubicin(34% succinoylation) conjugate with time; and

Figure 3 is a graphical representation of the preferential accumulation of succinoylated dextrin doxorubicin conjugate compared to an unconjugated control.

Detailed description of the invention

Example 1

Dextrin (Mw 51,000 Da) was succinoylated using a modification of the method described by Bruneel *et al* (Polymer, 35 (12),(1994), 2656-2658). Doxorubicin was then conjugated directly via an amide bond, conjugated via an N-*cis*-aconityl spacer or conjugated via a glycyl-N-*cis*-aconityl spacer.

Polymer degradation (unmodified dextrin, succinoylated dextrin (5, 15 mol %) and conjugate) was measured in the presence of amylase or lysosomal enzymes to monitor either changes in polymer molecular weight (GPC) or doxorubicin release (HLPC).

The dextrin-doxorubin conjugates had a doxorubicin loading of 6-12 wt% dependent on the reaction conditions used and the degree of succinoylation of the dextrin intermediate. Table 1 shows the characteristics of several batches of dextrin-succ-doxorubicin

Table 1 Characteristics of batches of dextrin-succ-doxorubicin

Batch No	Dox (wt%)	Free Dox (% total Dox)
1	11.7	0.8
2	11.9	2.0
3	8.7	1.2
4	8.4	0.1

After a 180 min incubation with amylase, unmodified dextrin is almost completely degraded to low molecular products, whilst the succinoylated dextrin (5 and 15 mol %) and dextrin-succ-doxorubicin show a biphasic pattern of degradation giving rise to fragments of Mw 4,000, 9,500 and 6,400 Da respectively. Unmodified dextrin had a $t_{½}$ (time for mass to reach half of its original) of 20 min, succinoylated dextrin and dextrin-succ-doxorubicin a $t_{½}$ of approximately 15 min.

Example 2

In this example the degradation of dextrins of different degrees of modification was compared. The results are shown in the accompanying drawing. It will be seen that native dextrin is rapidly degraded as are also dextrin with 5% succinoylation (whether with or without 6% Dox) and dextrin with 15% succinoylation. However, if dextrin is 34% succinoylated the degree of degradation is markedly less, there being zero% reduction of the peak mass of primary peak after 60 minutes and only 20% reduction after 180 minutes. In addition, Figure 2 shows that 34% succinoylated dextrin doxorubicin conjugate is similarly stable over an extended time course when compared to unconjugated or low level succinoylated (5%) controls.

Example 3

In this example increased uptake of 34% succinoylated dextrin-doxorubicin by tumour cells is shown. Male C57 were injected with 10^6 B16F10 murine melanoma cells subcutaneously with either doxorubicin hydrochloride or dextrin- succinoyl-

doxorubicin (34 mol % succinoylation, 11.8% doxorubicin) at 5mg/kg doxorubicin equivalence into the intrapertinoneal cavity (i.p.).

The mice were then culled after 2, 5, and 30 mins and after 1, 2, 5, 24, and 48 hours. Tumours were removed and weighed. The tumour was then homogenised and doxorubicin extracted and quantified by HLPC for total doxorubicin present, Figure 3.

Figure 3 shows there is approximately a three fold increase in tumour levels of doxorubicin were found for the conjugate for all time intervals from 2 min up to 24 hours. After this period, there is no difference between conjugate or the free drug. The elevated levels of the conjugate were at their highest 5 min after injection.

Example 4

In this example the pharmacology of succinolyated dextrin doxorubicin is determined and is presented in Table 2. Twenty four C57 black mice were injected subcutaneously (s.c.) with 10^5 B16F10 murine melanoma cells as described above and then monitored daily for well-being and the presence of palpable tumours. When the tumours were palpable, mice were randomly assigned into groups of six and their tumours measured with a micrometer gauge. Tumour size and mouse body weight is recorded. Each group is then injected intra-peritoneally with either sterile saline (negative control), free doxorubicin (5mg kg^{-1}) in sterile saline or dextrin-doxorubicin (11.8 wt%, 34% succinolyation) at either 5mg kg^{-1} or 10mg kg^{-1}, on days 0,1 and 2. The mice were monitored daily and tumour size and body weight recorded. Once the tumour area exceeded 2.89 cm^2 the mice were culled according to UKCCCR guidelines. Mouse survival is then expressed as % T/C (test/control saline).

The animals treated with doxorubicin (5mg kg^{-1}) displayed a drop in body weight consistent with toxicity. However all mice tolerated the dextrin –doxorubicin conjugate at both doses. The higher dose (10mg kg^{-1}) equates to approximately 2 mg

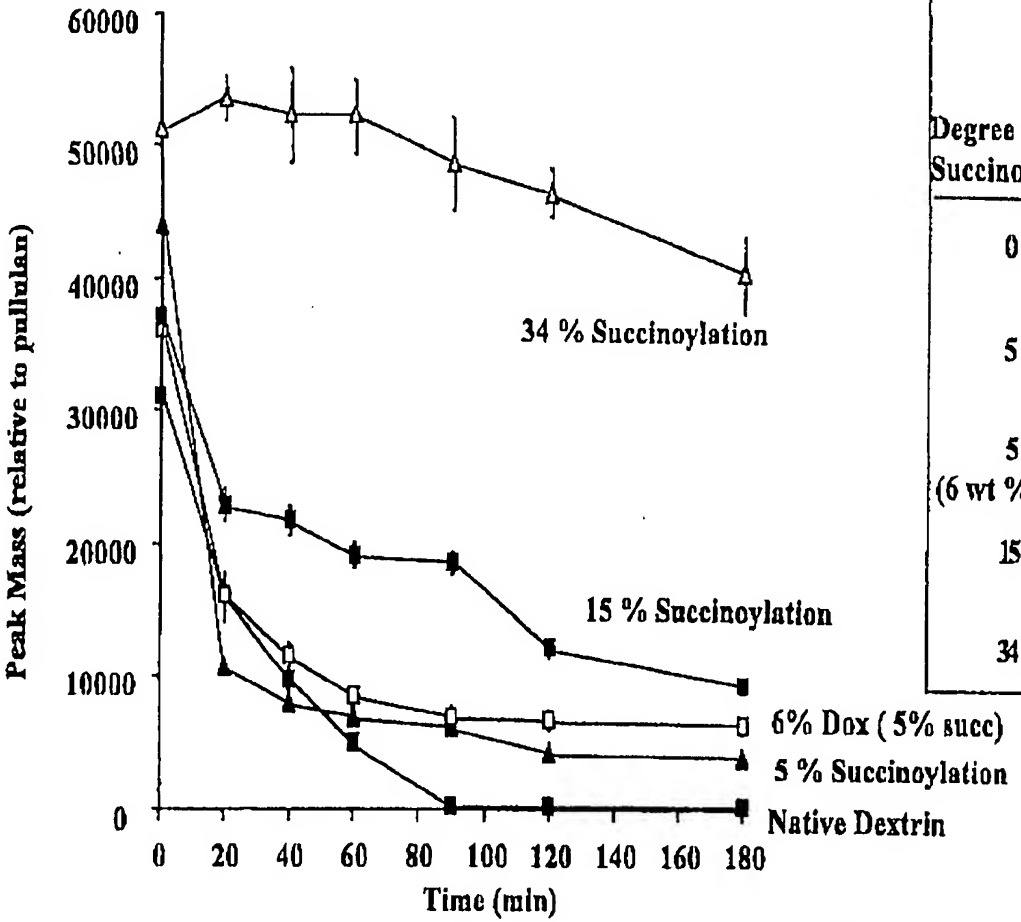
of conjugate. As shown in Table 2, dextrin- doxorubicin conjugate resulted in a T/C of approximately 140% indicating anticancer activity. In contrast, free doxorubicin was not active in this experiment.

Table 2

Compound	Dose mg kg^{-1} (day 0,1,2)	Days Survival after treatment (mean ± S.D)	T/C (%)	Toxic deaths
Control (saline)	-	4.3 ± 0.5	100	0/6
Doxorubicin	5	4.5 ± 0.5 ^{ns}	103	0/6
Dextrin-Dox	5	6.2 ± 0.8*	142	0/6
Dextrin-Dox	10	6.0 ± 1.1**	138	0/6

n=6 ns= not significant *p=0.0004 ** p=0.005

Degradation of Dextrins of different degrees of modification by α–Amylase



Degree of Succinoylation	% Reduction of peak mass of primary peak	
	60 min	180 min
0	84	99
5	85	90
5 (6 wt % Dox)	77	92
15	49	75
34	0	20

n=3 ±S.D.

- Dextrin degradation by amylase is dependent on the degree of backbone succinoylation. The dextrin-dox conjugate is rapidly degraded to oligomers

Figure 1

Figure 2

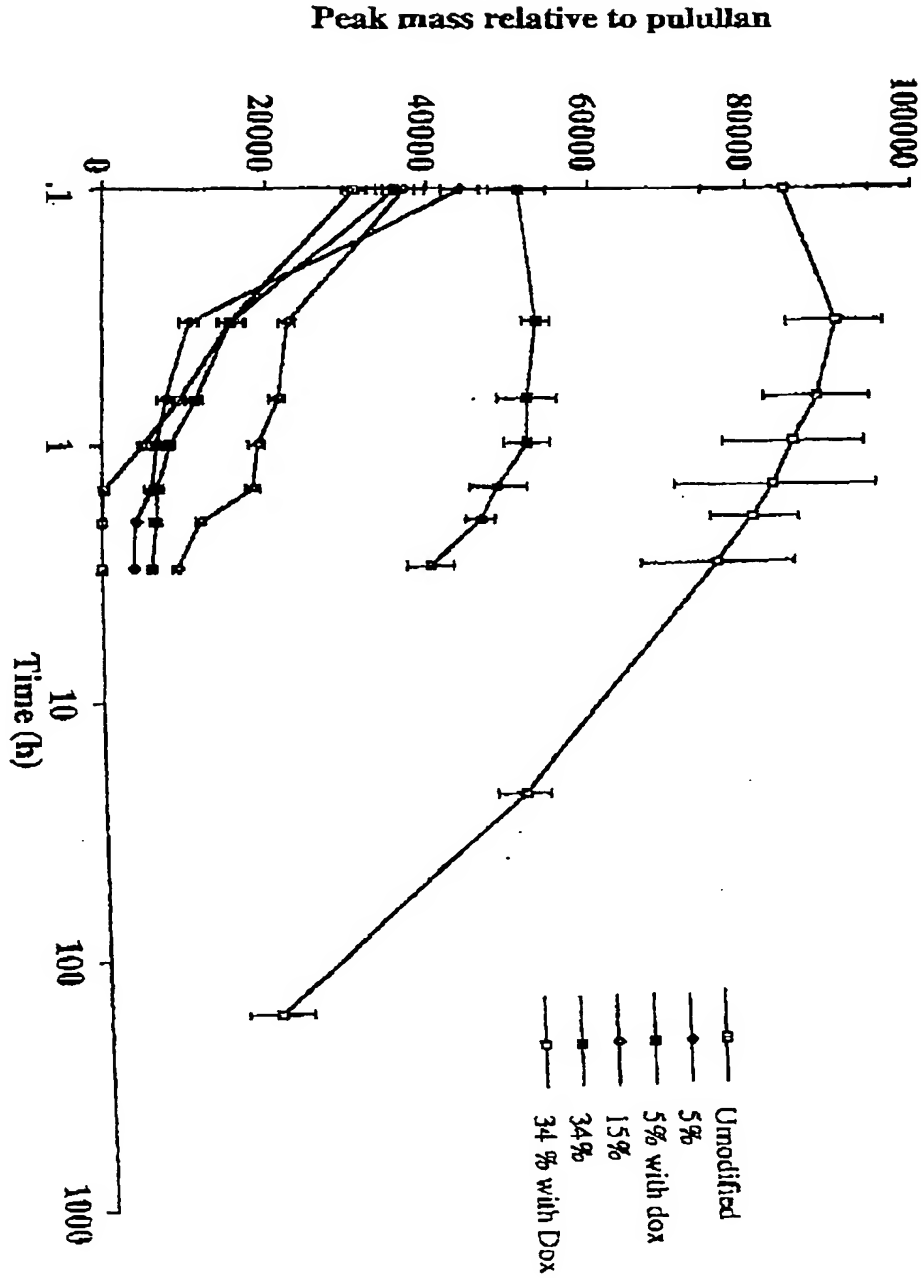


Figure 2. Degradation profiles of various succinoylated dextrins showing the degradation of 34% succinoylated dex-dox conjugate extended over 1 week at 37°C

Figure 3

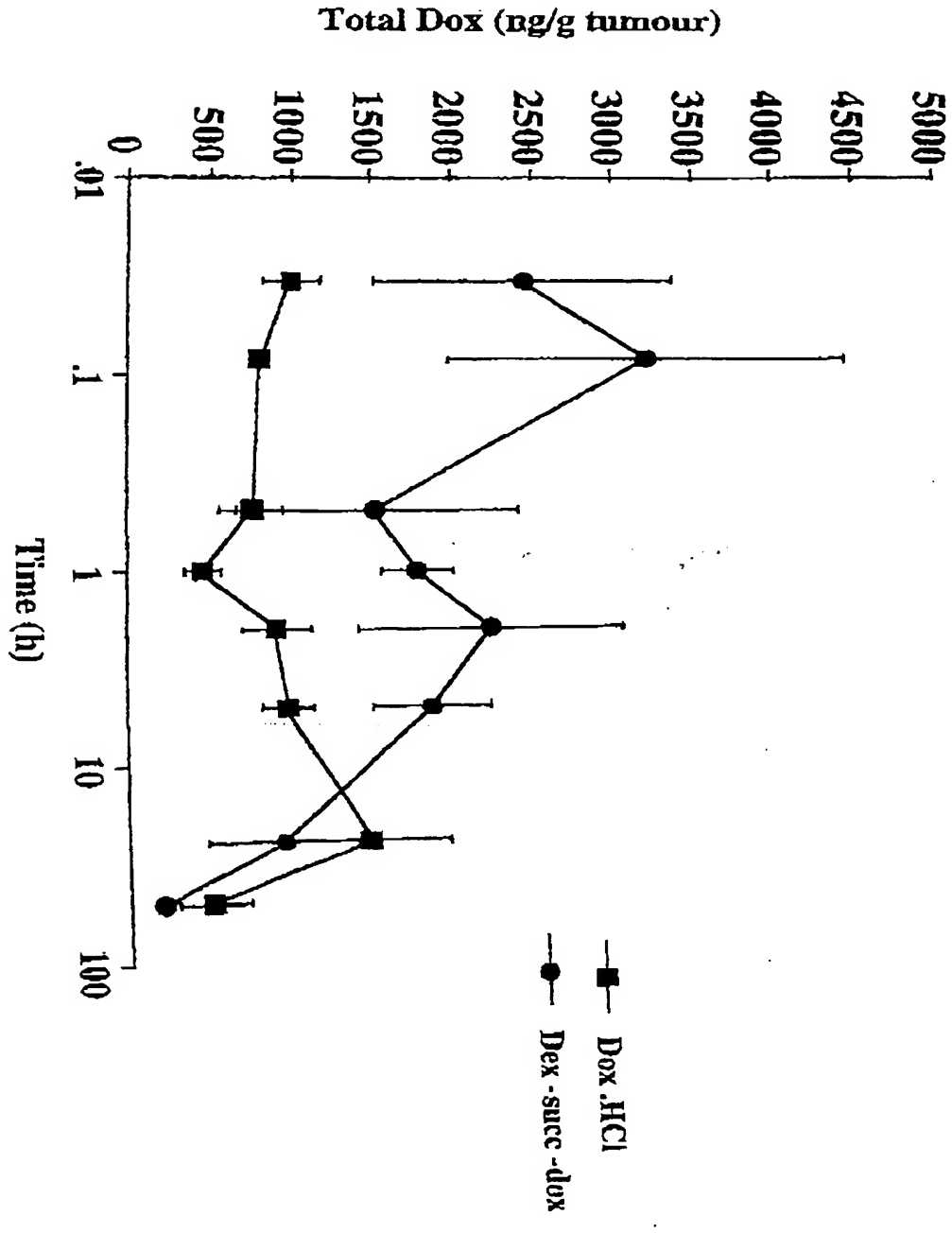


Figure 3. Pharmacokinetic profile of doxorubicin and dextrin-doxorubicin. Data shown is mean ± SE, n=3.

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Harrison Goddard Foote

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